Generation and characterization of an endogenously tagged SPG11-human iPSC line by CRISPR/Cas9 mediated knock-in

Krumm, Laura ; Pozner, Tatyana ; Kaindl, Johanna ; Regensburger, Martin ; Günther, Claudia ; Turan, Soeren ; Asadollahi, Reza ; Rauch, Anita ; Winner, Beate

Abstract: Pathogenic bi-allelic variants in the SPG11 gene result in rare motor neuron disorders such as Hereditary Spastic Paraplegia type 11, Charcot-Marie Tooth, and Juvenile Amyotrophic Lateral Sclerosis-5. The main challenge in SPG11-linked disease research is the lack of antibodies against SPG11 encoded spatacsin. Here, we describe the CRISPR/Cas9 mediated generation and validation of an endogenously tagged SPG11- human iPSC line that contains an HA tag at the C-terminus of SPG11. The line exhibits multi-lineage differentiation potential and holds promise for studying the role of spatacsin and for the elucidation of SPG11-associated pathogenesis. Resource Table.

DOI: https://doi.org/10.1016/j.scr.2021.102520

Stem Cell Research 56 (2021) 102520

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Genetically-Modified Single Cell Line

Generation and characterization of an endogenously tagged *SPG11*-human iPSC line by CRISPR/Cas9 mediated knock-in

Laura Krumm [a,1], Tatyana Pozner [a,1,*], Johanna Kaindl [a], Martin Regensburger [a,b,c], Claudia Günther [d], Soeren Turan [a,e], Reza Asadollahi [f], Anita Rauch [f], Beate Winner [a,c,*]

[a] Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Department of Stem Cell Biology, 91054 Erlangen, Germany
[b] Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Department of Molecular Neurology, 91054 Erlangen, Germany
[c] Center of Rare Diseases Erlangen (ZSEER), FAU Erlangen-Nürnberg, 91054 Erlangen, Germany
[d] Department of Medicine, Friedrich-Alexander-University Erlangen-Nürnberg, 91054 Erlangen, Germany
[e] Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Institute of Biochemistry, 91054 Erlangen, Germany
[f] Institute of Medical Genetics, University of Zürich, 8952 Schlieren-Zürich, Switzerland

A B S T R A C T

Pathogenic bi-allelic variants in the *SPG11* gene result in rare motor neuron disorders such as Hereditary Spastic Paraplegia type 11, Charcot-Marie Tooth, and Juvenile Amyotrophic Lateral Sclerosis-5. The main challenge in *SPG11*-linked disease research is the lack of antibodies against *SPG11* encoded spatacsin. Here, we describe the CRISPR/Cas9 mediated generation and validation of an endogenously tagged *SPG11*- human iPSC line that contains an HA tag at the C-terminus of *SPG11*. The line exhibits multi-lineage differentiation potential and holds promise for studying the role of spatacsin and for the elucidation of SPG11-associated pathogenesis.

Resource Table:

1. Resource Table:

Unique stem cell line identifier	TMOi001-A-4 https://hpscreg.eu/cell-line/TMOi001-A-4
Alternative name(s) of stem cell line	SPG11-HA
Institution	Department of Stem Cell Biology Universitätsklinikum Erlangen
Contact information of the reported cell line distributor	Holger Wend Email: Holger.Wend@uk-erlangen.de
Type of cell line	iPSC
Origin	Human
Additional origin info *(applicable for human ESC or iPSC)*	Female
Cell Source	CD34 + cord blood
Method of reprogramming	episomally
Clonality	Clonal
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	negative Southern blot originally reported for the parental line
Cell culture system used	N/A
Type of Genetic Modification	HA tag insertion
Associated disease	N/A
Gene/locus	SPG11
Method of modification/site-specific nuclease used	CRISPR/Cas9
Site-specific nuclease (SSN) delivery method	Plasmid transfection
All genetic material introduced into the cells	pCAG-SpCas9-GFP-U6-gRNA plasmid, single-stranded oligonucleotide donor DNA (ssODN)
Analysis of the nuclease-targeted allele status	N/A
Method of the off-target nuclease activity surveillance	Targeted PCR/sequencing
Name of transgene	N/A
Eukaryotic selective agent resistance (including inducible/gene expressing cell-specific)	N/A
Inducible/constitutive system details	N/A
Date archived/stock date	12/08/2019
Cell line repository/bank	N/A
Ethical/GMO work approvals	The original iPSC line was obtained from Thermo Fisher scientific. Ethical approval information for the

(continued on next column)

(continued)

(continued on next page)

* Corresponding authors at: Glückstraße 6, 91054 Erlangen, Germany.
E-mail addresses: tatyana.pozner@outlook.com (T. Pozner), Beate.winner@fau.de (B. Winner).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.scr.2021.102520
Received 21 July 2021; Accepted 21 August 2021
Available online 26 August 2021

(*continued*)

	parental line is listed at hpscreg: https://hpscreg.eu/cell-line/TMOi001-A
Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	Addgene plasmid # 79,144; http://n2t.net/addgene:79144; RRID: Addgene_79144

Manuscript section expected contents clarification

2. Resource utility

The bi-allelically endogenously tagged *SPG11*-human iPSC line enables precise detection of the *SPG11*-encoded spatacsin by employing antibodies against HA tag. Thus, it solves the previous problem of missing specific IP and ChiP grade antibodies against *SPG11*. The presented line can be further differentiated to study the role of spatacsin in different cell types and particularly during neural development.

3. Resource details

Bi-allelic pathogenic variants in *SPG11*, encoding spatacsin result in rare motor neuron disorders such as Hereditary Spastic Paraplegia type 11 (SPG11-HSP), Charcot-Marie Tooth disease, and Juvenile Amyotrophic Lateral Sclerosis-5 (ALS5). SPG11-HSP is the most frequent complicated autosomal-recessive HSP. Besides spasticity and paraparesis of the lower extremities, patients with SPG11-HSP present with additional symptoms such as cognitive decline, upper limb weakness, and peripheral neuropathy (Pozner et al., 2020).

SPG11 encodes a ~280 kDa protein, termed spatacsin that is involved in the function of the autophagic lysosomal machinery and vesicular trafficking. However, due to a lack of specific antibodies, the exact function of spatacsin is yet unclear. To overcome this obstacle we generated an endogenously tagged *SPG11* human-induced-pluripotent-stem-cell (hiPSC) line (SPG11-HA). The tagging was performed by employing CRISPR/Cas9 technology to insert an HA tag with homology-directed repair in a commercially available human episomal iPSC line (A18945; Thermo Fisher Scientific).

The cells were nucleofected with a vector coding for SpCas9, GFP, and a single guide-RNA (gRNA; addgene) targeting the end of the last exon of *SPG11* together with a single-stranded oligonucleotide (ssODN) donor DNA. The ssODN contained an HA coding region flanked by ~66–67 nucleotide homology arms (Fig. 1A-B). Following a single-cell sorting procedure and clone expansion, the positive candidates were identified by PCR amplification and Sanger Sequencing (Fig. 1A). The genotype was confirmed by Sanger and Amplicon/NGS sequencing (Fig. 1A,C). No pathogenic variants were detected at the predicted off-target loci (Fig. S1A). Chromosomal microarray analysis did not reveal any *de novo* copy number variations (CNV) in the nucleofected reporter line in comparison to iPSC control cells (ctrl) that did not undergo the CRISPR process (Fig. 1D). However, a gain at 20q11.21 was found in both cell lines. This CNV is recurrently found in hiPSCs and cancers, suggesting that it confers a proliferative or survival advantage (Nguyen et al., 2014). Both ctrl and SPG11-HA iPSC exhibited typical pluripotency cell-like morphology and were tested negative for mycoplasma (Fig. S1B-C). Both cell lines expressed pluripotency markers and differentiated in derivates of all three germ layers when tested with trilineage differentiation paradigm (Fig. 1E-F; Fig. S1D; Table 1).

To validate the functionality of the reporter line, expression of tagged spatacsin was investigated by Western blot. As expected, HA-tagged spatacsin was detectable at a size of 280 kDa (Fig. 1G). Due to the range of neurological symptoms exhibited upon loss of function of spatacsin, the neural differentiation capacity was assessed. Nearly 90% of differentiated control and SPG11-HA neural progenitor cells (NPCs) expressed both NPC markers SOX2 and Nestin (Fig. 1SE), rendering it a valid resource for studying neuronal phenotypes. Furthermore, HA-tagged spatacsin expression was also observed in SPG11-HA NPCs (Fig. S1F).

4. Materials and methods

4.1. Cell culture

Human Episomal iPSCs A18945 (Thermo Fisher Scientific) were cultured in mTeSR (STEMCELL Technologies) with 1% Penicillin/Streptomycin (Life Technologies) at 37 °C with 5% CO2 on Geltrex™ (500 μg for 57 cm^2, Thermo Fisher Scientific) coated plates. Cells were passages as clumps at a ratio of 1:3–1:10 every 3–5 days using Gentle Cell Dissociation Reagent (STEMCELL Technologies) without any survival promoter. Cells were received at passage 40 (P) and all experiments were conducted using passages numbers of P + 5 - P + 10 after genome editing. Mycoplasma testing was performed using MycoAlert™ Mycoplasma Detection Kit (Lonza).

4.2. Genome editing

For the generation of an *SPG11*-reporter line, an adapted version of a previously described protocol (Turan et al., 2019) was used. Briefly, the single gRNA spacers were selected using the CRISPOR tool (http://www.crispor.tefor.net) and cloned into pCAG-SpCas9-GFP-U6-gRNA plasmid (Addgene plasmid #79144). iPSCs were nucleofected with 5 μg pCAG-SpCas9-GFP-U6-gRNA plasmid and 0.2 nM Donor DNA Oligonucleotide (IDT) using the nucleofector 2B (program B-16) and the human stem cell nucleofector kit 2 (Lonza; Cat.# VPH-5022). To increase the homologous recombination efficiency, the cells were treated with a 20 μmol HDR enhancer (IDT; Cat.#1081072) for 24 h. After an additional 24 h, single GFP + iPSCs were sorted into a Geltrex (Thermo Fisher Scientific) coated 96-well plate containing CloneR (10% in mTeSR, STEMCELL Technologies). 7–10 days later, clones were mirrored into two 48-well plates. One plate was cryopreserved using Bambanker (Nippon) and the remaining plate was used for genomic DNA (gDNA) extraction using QIAamp DNA Blood Mini Kit (Qiagen). To validate the tag integration, exon 40 of *SPG11* was amplified using Q5®High-Fidelity DNA Polymerase (New England Biolabs). Since the reverse primer was complementary to the HA tag intersection, only exon 40 including HA was amplified (for primer sequence see Table 2). Flanking PCR to sequence the complete region of interest served as additional confirmation (Table 2). For NGS-based genotyping of the *SPG11*-reporter line, the Amplicon-EZ (GENEWIZ) was used (for primers see Table 2).

4.3. Off-target analysis

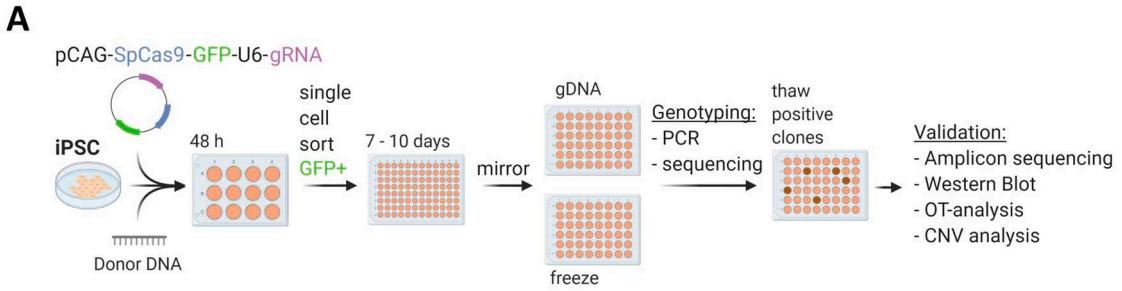
The four highest-scoring predicted exonic off-target regions identified by CRISPOR (http://www.crispor.tefor.net) were examined according to a previous description (Pozner et al., 2018).

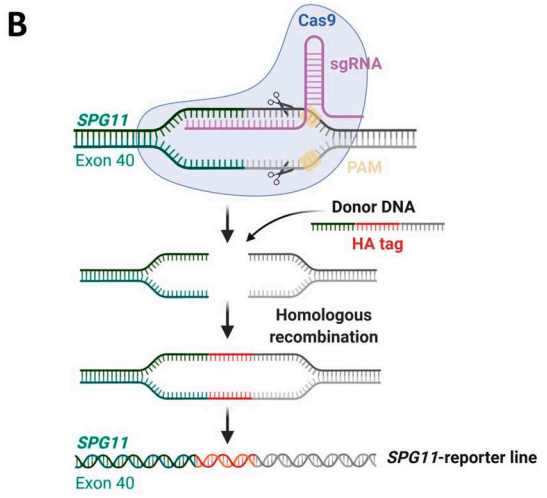
Flow cytometry, immunocytochemistry and western blot

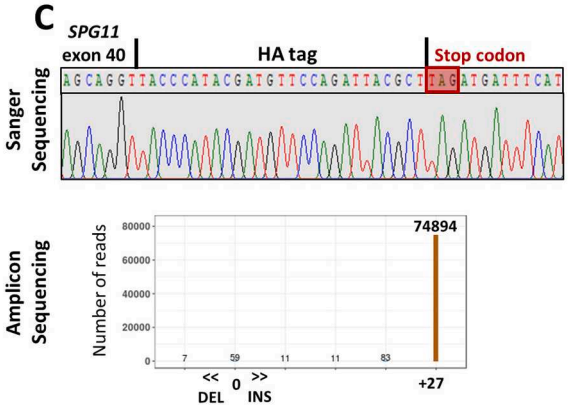
Flow cytometry and immunohistochemistry analysis were performed as previously described (Boerstler et al., 2020) (for antibodies see Table 2). Western blot was performed similarly to described conditions (Pérez-Brangulí et al., 2019).

4.4. Chromosomal microarray analysis

Extracted gDNA from ctrl and SPG11-HA iPSCs was analyzed with Affymetrix CytoScan HD (Affymetrix Inc., Santa Clara, CA, USA) and the data set of samples were evaluated with Affymetrix Chromosome

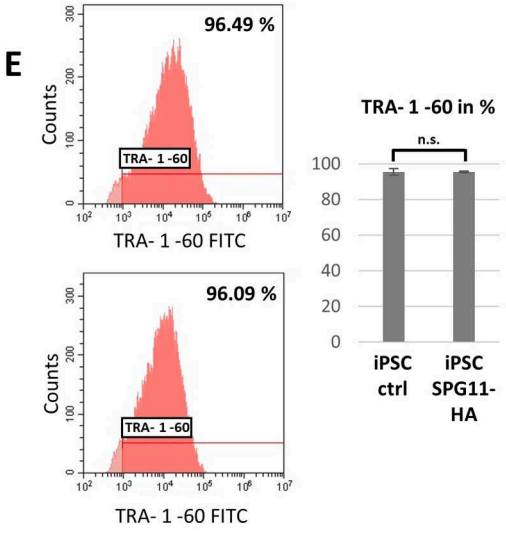


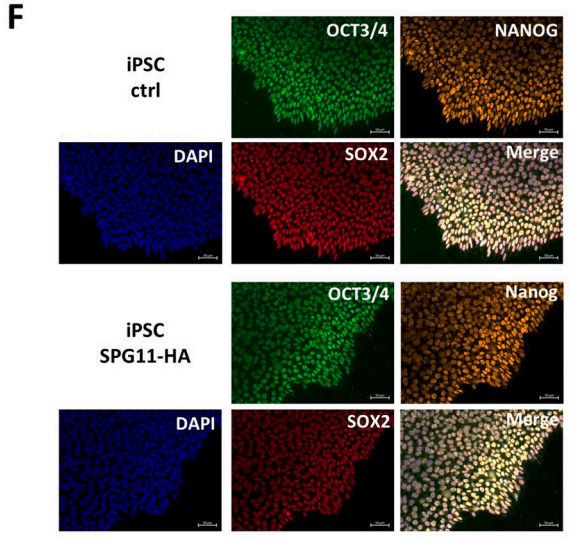


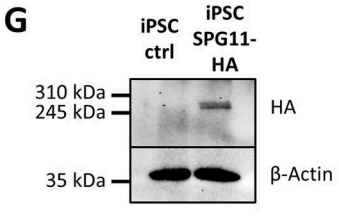


D

Line	iPSC ctrl	iPSC SPG11-HA
CNV 1 GRCh37 /hg19	20q11.21 31001699_29652121 (gain)	20q11.21 31001699_29838604 (gain)
CNV 2 GRCh37 /hg19	7q33 133213839_132638934 (gain)	7q33 133200522_132634639 (gain)







(caption on next page)

Fig. 1. . A. Paradigm depicting the genome editing strategy. The iPSCs are nucleofected with a plasmid expressing gRNA and SpCas9-GFP. After 48 h in 12-well plate, the GFP positive cells are single-cell sorted into 96-well plate for 7–10 days expansion. Afterwards, the cells are mirrored into 48-well plates for further genotyping and cryopreservation. Following the genotyping of the clones by performing PCR and sequencing, candidate clones are thawed and validated. OT = off-target; CNV = copy number variation. B. Schematic representation of the CRISPR/Cas9 mediated targeting of the last exon of the *SPG11* gene. C. Results of the Amplicon-EZ NGS-based sequencing depicting the incorporated sequence of HA tag into the C-terminal domain of *SPG11* (top), and demonstration that 74,894 reads out of 80,000 reads contained the tag (bottom). D. Chromosomal microarray analysis of the analysed lines presenting the detected CNVs. E. Pluripotency analysis by using Tra 1–60 flow cytometry analysis. F. Immunofluorescence images of the gene-edited clone (SPG11-HA) and control cells (ctrl) demonstrating comparably high expression of pluripotency markers NANOG, OCT3/4 and SOX2 (scale bar = 50 μm). G. Immunoblot using an antibody against HA, reveals a precise and specific detection corresponding to the size of spatacsin and HA tag (~280 kDa).

Table 1
Characterization and validation.

Classification (optional *italicized*)	Test	Result	Data
Morphology	Photography	Visual record of line: normal	Fig. S1C
Pluripotency status evidence for the described cell line	Qualitative analysis *(i.e. Immunocytochemistry, western blotting)*	Staining of pluripotency markers: Oct 3/4, Nanog, Sox2	Fig. 1F
	Quantitative analysis *(i.e. Flow cytometry, RT-qPCR)*	Flow cytometry analysis of cell surface marker Tra 1-60	Fig. 1E
Karyotype	Karyotype (G-banding) and higher-resolution, array-based assays (KaryoStat, SNP, etc.)	Normal karyotype according to G-banding was reported for the parental cell line. SPG_HA line was evaluated with higher resolution analysis: Affymetrix CytoScan HD Resolution > 20 kb	Fig. 1D
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR across the edited site or targeted allele-specific PCR	Bi-allelic HA tag insertion	Fig. 1C
	Transgene-specific PCR	N/A	N/A
Verification of the absence of random plasmid integration events	PCR/Southern	Negative Southern Negative PCR	Reported for the parental line submitted in archive with journal
Parental and modified cell line genetic identity evidence	STR analysis, microsatellite PCR (mPCR) or specific (mutant) allele seq	STR Analysis: 16 sites tested and all the loci matched the parental line (ctrl)	submitted in archive with journal
Mutagenesis / genetic modification outcome analysis	Sequencing (genomic DNA PCR or RT-PCR product) PCR-based analyses Southern Blot or WGS; western blotting (for knock-outs, KOs)	Amplicon-EZ NGS-based sequencing	Fig. 1C
Off-target nuclease analysis-	PCR across top 5/10 predicted top likely off-target sites, whole genome/exome sequencing	Top predicted likely-off target sites	Fig. S1A
Specific pathogen-free status	Mycoplasma	Mycoplasma testing by luminescence: all negative	Fig. S1C
Multilineage differentiation potential	e.g. Embryoid body formation OR Teratoma formation OR Scorecard OR Directed differentiation	Directed differentiation	Fig. S1D
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype - additional histocompatibility info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Analysis Suite (ChAS v3.1.0.15) as previously described (Asadollahi et al. 2014). We assessed mosaic and non-mosaic copy number variants (CNVs) sizing > 20 kb for their overlap with annotated exons and their presence in control databases. Rare CNVs > 20 kb affecting exonic regions were considered for their pathogenicity. The two samples were compared for newly occurring CNVs after the CRISPR process.

4.5. Trilineage differentiation

Human germ layer differentiation was performed using the STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies) according to manufacturer's guidelines. Immunohistochemistry staining of the specific germ layer markers was performed using Human Three Germ Layer 3-Color Immunohistochemistry Kit (R&D Systems).

4.6. Differentiation into neural progenitor cells

Neural differentiation was performed using a modified version of a previously described protocol (Turan et al., 2019). NPCs were split once per week using Accutase (Thermo Fisher Scientific).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Primary antibody	Rabbit Anti-HA tag	1:500	Abcam Cat# ab9110; RRID: AB_307019_307019
Primary antibody	Mouse Anti-Adaptin γ	1:1000	BD Bioscience Cat# 610385, RRID: AB_397768
Primary antibody	Mouse Anti-beta-Actin	1:5000	Sigma-Aldrich Cat# A1978, RRID: AB_476692
Pluripotency marker	Rabbit Anti-Sox2	1:500	Cell Signalling Cat# 3579, RRID: AB_2195767
Pluripotency marker	Goat Anti-Nanog	1.500	R&D Systems Cat# AF1997, RRID: AB_355097
Pluripotency marker	Mouse Anti-Oct-3/4 (C-10)	1:500	Santa Cruz Cat# sc-5279, RRID: AB_628051
Pluripotency marker	Mouse Anti-Tra 1-60-R Alexa 488	1:20	Biolegend Cat# 330614, RRID: AB_2119064
Differentiation marker	Mouse Anti Nestin-PerCP-Cy	1:50	BD Bioscience Cat#561231, RRID: AB_10562199
Differentiation marker	Mouse Anti-Sox2-PE	1:50	BD Bioscience Cat#561610, RRID: AB_10712763
Secondary antibody	Alexa Fluor™anti-mouse 488	1:500	Thermo Scientific Cat#A32766, RRID: AB_2762823
Secondary antibody	Alexa Fluor™anti-goat 546	1:500	Thermo Scientific Cat#A11056, RRID: AB_2534103
Secondary antibody	Alexa Fluor™anti-rabbit 647	1:500	Thermo Scientific Cat#A32794, RRID: AB_2762835
Secondary antibody	Anti-mouse HRP	1:10000	Thermo Scientific Cat# A16017, RRID: AB_2534691
Secondary antibody	Anti-rabbit HRP	1:5000	Thermo Scientific Cat#A16035, RRID: AB_2534709
Site-specific nuclease			
Nuclease information	SpCas9		
Delivery method	Nucleofection		
Selection/enrichment strategy	Single-cell sorting		
Primers and Oligonucleotides used in this study			
	Target	Forward/Reverse primer (5′-3′)	
e.g. Episomal Plasmids (qPCR or RT-PCR)	e.g. OCT4 Plasmid	N/A	
e.g. Pluripotency Markers (qPCR)	e.g. NANOG, etc.	N/A	
e.g. House-Keeping Genes (qPCR)	e.g. HSP90AB1, etc.	N/A	
e.g. Genotyping (desired allele/transgene presence detection)	PCR specific for the targeted allele	N/A	
Targeted mutation analysis/sequencing	Flanking PCR primer	CAAGACTGCTCCTCTGCAC/	
	Junction PCR primer	TGCTACCTACTACCCACAAAGG	
	Amplicon PCR Primer with **adapter sequence**	CAAGACTGCTCCTCTGCAC/ GGAACATCGTATGGGTAACCTG **ACACTCTTTCCCTACACGACGCTCTTCCGATCTC** AAGACTGCTCCTCTGCAC/ **GACTGGAGTTCAGACGTGTGCTCTTCCGATCTT** GCTACCTACTACCCACAAAGG	
gRNA oligonucleotide/crRNA sequence	gRNA oligonucleotide sequence	caccgAGCAGGTTAGATGATTTCAT/ aaacATGAAATCATCTAACCTGCTc	
Genomic target sequence(s)	Including PAM and other sequences likely to affect UCN activity	APE file submitted in archive with journal	
Top off-target mutagenesis predicted site sequencing (for CRISPR/Cas9 and TALENs) primers	OT1- L3MBTL1	GGCATAGTGGTGCCTGAAGT/	
	OT2- RP11-673C5.2	TGGCAGTGACTGGAAG (379 bp)	
	OT3- RNF216P1	TTCTGTCCTGCTGCACATTC/	
	OT4- AC087645.1/BIRC5	TTTGCTTTGTCCTGCAGATG (444 bp)	
		GGCTGCTTTTCAACCAAGAG/	
		CCGTGGACCTTGACTGATTT (309 bp)	
		TTTCTGCCACATCTGAGTCG/	
		TGTCGAGGAAGCTTTCAGGT (391 bp)	
pCAG-SpCas9-GFP-U6-gRNA plasmid specific primers to verify lack of genomic integration	pCAG-A	cccacgttctgcttcactct/tcacgcagccacagaaaaga (448 bp)	
	pCAG-B	CCACGACGGAGACTACAAGG/	
	pCAG-C	CCGCTCGTGCTTCTTATCCT (448 bp)	
	pCAG-D	CAGAGCTTCATCGAGCGGAT/	
		CGAACAGGTGGGCATAGGTT (466 bp)	
		GAACCAGACCACCCAGAAGG/	
		CTCGGCCTTGGTCAGATTGT (415 bp)	
ODNs/plasmids/RNA templates used as templates for HDR-mediated site-directed mutagenesis.	ssODN	TCTTCTCATCACATCTGTCAGAATCTGCTAACAGTAC AAGAAAACAGACACCTATGAAATCATCTACTTGTCG TCGTCGTCCTTGTAGTCGATGTCGTGGTCCTTGTAGT CACCGTCGTGGTCCTTGTAGTCACCTGCTAGCATGTC CTTTAGACAGCAACCTGTCTGAGGGTCCTTCAGAAG CACATTTACAATTTCAT	

Acknowledgements

The work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - 270949263/GRK2162 (to L.K., T. P., J.K., M.R., S.T., and B.W.), by the German Federal Ministry of Education and Research funded treatHSP consortium (01GM1905B to B.W. and M.R. and grants 01GQ113, 01GM1520A, 01EK1609B to B.W.), and by the Bavarian Ministry of Education and Culture, Science and the Arts within the framework of the Bavarian Network for studying brain cell interactions: ForInter. Additionally the work was supported by the Tom Wahlig Stiftung (to T.P. and M.R.).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102520.

References

Boerstler, T., Wend, H., Krumbiegel, M., Kavyanifar, A., et al., 2020. CRISPR/Cas9 mediated generation of human ARID1B heterozygous knockout hESC lines to model Coffin-Siris syndrome. Stem Cell Res 47, 101889. https://doi.org/10.1016/j.scr.2020.101889.

Nguyen, HT, Geens, M, Mertzanidou, A, Jacobs, K, Heirman, C, Breckpot, K, et al., 2014. Gain of 20q11.21 in human embryonic stem cells improves cell survival by increased expression of Bcl-xL. 2014. Mol Hum Reprod 20, 168–177. https://doi.org/10.1093/molehr/gat077.

Pérez-Brangulí, F., Buchsbaum, I.Y., Pozner, T., Regensburger, M., Fan, W., Schray, A., et al., 2019. Human SPG11 cerebral organoids reveal cortical neurogenesis impairment. Hum Mol Genet 28. https://doi.org/10.1093/hmg/ddy397.

Pozner, T., Regensburger, M., Engelhorn, T., Winkler, J., Winner, B., 2020. Janus-faced spatacsin (SPG11): involvement in neurodevelopment and multisystem neurodegeneration. Brain. https://doi.org/10.1093/brain/awaa099.

Pozner, T., Schray, A., Regensburger, M., Lie, D.C., Schlötzer-Schrehardt, U., Winkler, J., et al., 2018. Tideglusib Rescues Neurite Pathology of SPG11 iPSC Derived Cortical Neurons. Front Neurosci. https://doi.org/10.3389/fnins.2018.0091410.3389/fnins.2018.00914.s001.

Turan, S., Boerstler, T., Kavyanifar, A., Loskarn, S., Reis, A., Winner, B., et al., 2019. A novel human stem cell model for Coffin-Siris syndrome-like syndrome reveals the importance of SOX11 dosage for neuronal differentiation and survival. Hum Mol Genet 28, 2589–2599. https://doi.org/10.1093/hmg/ddz089.